

AMENDMENTS TO THE CLAIMS

1. (Currently Amended) An isolated nucleic acid comprising a nucleotide sequence selected from the group consisting of:

(A) a nucleotide sequence encoding a polypeptide having the amino acid sequence of SEQ ID NO: 14, wherein the polypeptide possesses a ceramidase activity;

(B) a nucleotide sequence comprising SEQ ID NO: 15, wherein said nucleotide sequence encodes a polypeptide possessing a ceramidase activity;

(C) a nucleotide sequence which hybridizes to the complement of the nucleotide sequence of SEQ ID NO: 15 ~~(A) or (B)~~ under stringent conditions, wherein said stringent conditions comprise 7% PEG 6000 containing 10% SDS solution at 60°C, and washing three times with 2xSSC containing 0.1% SDS for 15 minutes at 60°C of 6 x SSC containing 0.5% SDS, 5 x Denhardt's, and 100 µg/ml salmon sperm DNA at 50°C, wherein said nucleotide sequence encodes a polypeptide having activity of hydrolyzing any one of substances selected from the group consisting of (i) N-Lauroylsphingosine, (ii) N-Palmitoylsphingosine, (iii) N-Stearoylsphingosine, (iv) N-Palmitoylsphinganine, (v) N-Stearoylsphinganine, and (vi) 12-((N-(7-nitrobenz-2-oxa-1,3-diazol-4-

yl) amino)dodecanoyl sphingosine possessing a ceramidase activity; and

(D) a nucleotide sequence different from the nucleotide sequence of any one of the above (A) to (C) due to via degeneracy of the genetic code, wherein said nucleotide sequence encodes a polypeptide having activity of hydrolyzing any one of substances selected from the group consisting of

(i) N-Lauroylsphingosine, (ii) N-Palmitoylsphingosine,
(iii) N-Stearoylsphingosine, (iv) N-Palmitoylsphinganine,
(v) N-Stearoylsphinganine, and (vi) 12-((N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)dodecanoyl)sphingosine possessing a ceramidase activity.

2. (Currently Amended) The nucleic acid according to claim 1, wherein the ceramidase activity of the polypeptide is detected by the following steps:

(a) incubating an expression product in a reaction mixture comprising 550 pmol of 12-((N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)dodecanoyl)sphingosine and 1.0% (W/V) sodium cholate in 20 µl of 25 mM Tris-hydrochloric acid buffer (pH 7.5) at 37°C for 30 minutes; and

(b) detecting the formation generation of a 12-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)dodecanoyl acid in the reaction.

3. (Currently Amended) The nucleic acid according to claim 1 or 2, wherein the polypeptide exhibits at least the following characteristics:

- (i) action of hydrolyzing ceramide to generate sphingoid and a fatty acid;
- (ii) substrate specificity of hydrolyzing N-acylsphingosine, but not acting on galactosylceramide, sulfatide, Galb1-3GalNAcb1-4(NeuAca2-3)Galb1-4G1cb1-1'Cer (GM1a) GM1a, and sphingomyelin;
- (iii) optimum pH of from 7.0 to 8.0; and
- (iv) ~~wherein incubation no lowering of activity when treated in 20 mM Tris-hydrochloric acid (pH 7.5) containing 0.1% polidocanol at 37°C for 24 hours does not decrease activity of said polypeptide, whereas incubation in 20 mM Tris-hydrochloric acid (pH 7.5) containing 0.1% polidocanol, but lowering of activity to about 30% by a treatment at 60°C for 1 hour decreases activity of said polypeptide to about 30%.~~

4. (Previously Presented) A recombinant DNA comprising the nucleic acid of claim 1.

5. (Previously Presented) An expression vector comprising the nucleic acid of claim 1 or the recombinant DNA of claim 4.

6. (Currently Amended) A transformed cell ~~transformant~~ comprising the expression vector of claim 5.

7. (Previously Presented) A method for producing a polypeptide possessing a ceramidase activity, comprising the steps of culturing the transformant of claim 6 under conditions appropriate for expression of the polypeptide, and collecting a polypeptide possessing a ceramidase activity from the resulting culture.

8. (Previously Presented) An isolated polypeptide comprising the amino acid sequence of SEQ ID NO: 14, wherein said polypeptide possesses a ceramidase activity.

9. (Previously Presented) An isolated polypeptide possessing a ceramidase activity, wherein said polypeptide is encoded by the nucleic acid of claim 1.

10. (Currently Amended) The polypeptide according to claim 8 or 9, wherein the ceramidase activity is detected by the following steps:

(a) incubating an expression product in a reaction mixture comprising 550 pmol of 12-((N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)dodecanoyl)sphingosine and 1.0% (w/v) sodium cholate in 20 μ l of 25 mM Tris-hydrochloric acid buffer (pH 7.5) at 37°C for 30 minutes; and

(b) detecting the formation generation of a 12-((N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)dodecanoyl acid in the reaction.

11. (Currently Amended) An isolated antisense DNA which is complementary to the nucleic acid of claim 1.

12. (Currently Amended) An isolated antisense RNA which is complementary to the nucleic acid of claim 1.

13. (Currently Amended) An expression vector comprising the antisense DNA of claim 11.

14-19. (Cancelled).

20. (Currently Amended) A method of controlling an amount of ceramide in a cell ~~and/or in a tissue~~, comprising the step of introducing the nucleic acid of claim 1 or a complementary an antisense nucleic acid thereof into the cell ~~and/or in a tissue~~, thereby controlling the amount of ceramide in the cell ~~and/or in a tissue~~.

21. (Cancelled).